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PRIMER NOTE

Isolation and characterization of 149 novel microsatellite DNA markers for striped bass, *Morone saxatilis*, and cross-species amplification in white bass, *Morone chrysops*, and their hybrid

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Abstract

To support detailed genetic analysis of striped bass (*Morone saxatilis*) and white bass (*Morone chrysops*), we isolated 153 microsatellite loci from repeat-enriched striped bass DNA libraries. Of these, 147 markers amplified in striped bass (average 4.7 alleles per locus) and 133 in white bass (average 2.2 alleles per locus). One hundred twenty-two markers amplified in their hybrid. Development of new microsatellite markers will facilitate evaluations of genetic structure in wild populations and will support pedigree analysis and linkage mapping for selective breeding.

Keywords: aquaculture, hybrid, microsatellite DNA, *Morone*, striped bass

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The anadromous striped bass (*Morone saxatilis*) is native to coastal regions of eastern North America from Nova Scotia to Florida and within the Gulf of Mexico west to Louisiana. This species supported valuable commercial and recreational fisheries, but experienced significant population declines in the mid-1970s due to habitat degradation and overfishing. Population bottlenecks and supplementation of depleted stocks with non-native fish likely have altered the historic population genetic structure of striped bass. Additionally, widespread introductions of a fertile hybrid striped bass (HSB; white bass *Morone chrysops* × *Morone saxatilis*) for stock enhancement and recreational fishing may have permitted introgression with wild *Morone* species. Although genetic differentiation has been detected among several geographical strains of *M. saxatilis*, unusually low genetic variation appears to characterize this species (Waldman *et al*. 1988) and has limited the number of informative molecular markers available for detailed population genetic analysis.

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Numerous high-resolution molecular markers also are needed for pedigree tracking in aquacultured populations of *Morone* species. Declines in wild striped bass harvests created market demand for production of HSB as foodfish. Until recently, this industry remained largely dependent on wild fish for broodstock. Efforts towards sustainable aquaculture of HSB by domestication and selective breeding of the parent species are now underway and require highly polymorphic markers for progeny identification.

To date, 46 published microsatellite markers have been characterized for *Morone* species. Only 12 markers have ≥ 6 alleles in striped bass and four have ≥ 3 alleles for white bass (see review in Garber & Sullivan 2006). Additional informative markers are necessary not only to support detailed genetic analyses for conservation and management of wild populations, but also for selective breeding and linkage mapping in aquaculture.

Microsatellite markers were developed from a repeat-enriched striped bass DNA library using a protocol by Ostrander *et al*. (1992) modified by Westerman *et al*. (2005). Genomic DNA (100 μg) was extracted from whole blood from a striped bass and digested with *Sau*3A1 and *Bam*HI (Invitrogen). Three enriched libraries containing CA:GT

Table 1 Summary data for 29 of the 149 microsatellite loci successfully amplifying in striped bass (SB) and white bass (WB) with observed allele size ranges, number of individuals assayed (N), number of alleles observed (k), observed (H_O) and expected (H_E) heterozygosities (boldface numbers indicate significant deviation from HWE) and annealing temperatures (T_a) in °C

Locus	GenBank Accession no.	Primer sequences (5′–3′)	Repeat motif	Allele size range (bp) SB / WB	N/k SB / WB	H_O/H_E SB / WB	T_a
MSM1067	BV678238	F: GGAATCAAATCCCTGCTGTTATAATCT	$(CA)_{14}(GT)_{11}$	190–210	6/5	0.83/0.8	58
		R: CTATCTGGACTTTATCCCTACGAGTGA		154–159	33/2	0.71/0.49	
MSM1085	BV678171	F: TCTTTTATTTTTAGCCTCATTCAGACTGAT	$(CA)_{31}$	144–189	15/13	0.73/0.9	58
		R: CAGCAACAGATGATGGTCAAGTATG		109–111	6/2	0.33/0.3	
MSM1092	BV678175	F: CACTCTGGTTTACTGAATAAGCTCC	$(CA)_{28}$	183–216	15/7	0.64/0.79	58
		R: GTGCAGCCACAGTGTGTCTAC		152	6/1	—	
MSM1094	BV678177	F: TCCATCCCATCCTCTGTATC	$(CA)_{25}$	125–157	15/7	0.85/0.83	58
		R: GCCTCTCTGAGCTTATCCCTA		160–183	6/9	1/0.95	
MSM1095	BV678178	F: TGATAGCTGTGGTTACTGGTTG	$(TG)_{28}$	155–188	15/8	0.93/0.81	60
		R: AGGCTGATGCTGCAGTTATT		147–170	6/3	0.33/0.59	
MSM1096	BV678179	F: GACATGCACAGAGACAAATG	$(CA)_{25}$	182–200	15/7	0.73/0.8	60
		R: CAAGCTCCAGTCTATAACAGC		180	6/1	—	
MSM1107	BV678188	F: GATAACCTATAGGCCACGTTG	$(GT)_{13}$	144–225	15/8	0.6/0.76	60
		R: TTCACAAGACTGCACGTACA		129	6/1	—	
MSM1137	BV678209	F: GCAGGCAGGTTTTATCTAGGTTAG	$(CA)_{35}$	153–240	15/16	0.79/0.92	55
		R: ACACTCTCTGCCCTTTGAGTTC		125	6/1	—	
MSM1138	BV678652	F: GGCCACCTTCAACTAACATACTTC	$(TG)_{17}$	184–192	15/5	0.5/0.75	61
		R: CGCTCCGTGTCTTGTCTAAAT		159–167	6/3	0.67/0.62	
MSM1139	BV678210	F: TCTTTCCCAGCAGTGAACAAACTAT	$(AC)_{34}$	171–201	15/8	0.77/0.82	61
		R: GCTGTGGCCAAATTATTGTAGTCAG		166–172	6/3	0.17/0.44	
MSM1140	BV678211	F: GCCAAGCCATTGCATTATCCCATT	$(AC)_{17}$	179–209	15/8	0.6/0.78	61
		R: TCACTCCTCATGCCACTTTCGACC		158–178	6/5	0.67/0.79	
MSM1144	BV678214	F: CAGTGGGAGGGAGAGTAAATA	$(AC)_{25}$	115–150	15/10	0.92/0.88	61
		R: GCAGGATAGGAATCAGTCG		175–183	6/3	0.5/0.44	
MSM1145	BV678215	F: CTCCTCAAAATGTGTGACCC	$(CA)_{43}$	155–293	15/17	0.92/1.00	55
		R: TGCAGTGTTGATCAGGTTACAG		202–253	6/8	0.5/0.93	
MSM1152	BV678222	F: TGAACTACAGCCTATACCAGA	$(CA)_{23}$	197–249	15/10	**0.25/0.87**	61
		R: AGAGTCAAGAACCTTGTGG		179	6/1	—	
MSM1155	BV678224	F: GTGCTCGTACCTGAAAAGTACACATGC	$(CA)_{23}$	154–181	15/8	0.57/0.83	61
		R:CAGCCTAACAAATTAAACACCATTATGCAG		159–163	6/2	0.17/0.41	
MSM1157	BV678226	F: TGTCTGAGCAGGATGCTTACC	$(CA)_{34}$	165–200	15/12	0.67/0.9	64
		R: GCCCATTAGCTTTTGTAGCAAC		140	6/1	—	
MSM1161	BV678228	F: TTCGACCTCGCCAACTTC	$(CT)_{14}(CA)_{10}$	154–188	15/8	0.93/0.88	61
		R: TCGGGTTCTCTAAAGCTACCTG		171–175	6/3	0.2/0.6	
MSM1165	BV678232	F: TCGGTCAGAGTGAGCTCAGAGT	$(AC)_{50}$	213–231	15/6	0.57/0.8	61
		R: CAGGTTACAACGACCACGACA		160–162	6/2	0.33/0.3	
MSM1166	BV678233	F: CTGAGGTCTCAACACATTCAGT	$(CA)_{18}$	176–207	15/7	0.67/0.84	61
		R: TCAGTAACCAAACACTCCCTG		182	6/1	—	
MSM1168	BV678235	F: GAGAACGGAGCCGACATCA	$(CA)_{27}$	132–155	15/6	0.73/0.82	62
		R: CATGAAAATGGGTCCTATGGGA		141–143	6/2	0/.67	
MSM1186	BV678307	F: TATGGAGGTGGTTTAGGGTCT	$(CA)_{25}$	192–212	15/6	0.67/0.75	63
		R: TCAGGAGTTACAGAACGGAGA		192	6/1	—	
MSM1193	BV678264	F: ACTCAGTTACTCAACGCCCTC	$(CA)_{20}$	122–145	15/7	0.71/0.79	61
		R: CCACTGGGCTTTGTCTAACTC		130	6/1	—	
MSM1194	BV678265	F: CACATCAGCCTTCATTACCAC	$(GT)_{30}$	223–258	15/7	**0.33/0.78**	61
		R: TGTGAGCAATAAACTGATGCC		225–231	6/3	0.17/0.44	
MSM1208	BV678286	F: AACTCAAACTGCAGCGTTCTC	$(TA)_{31}$	171–195	15/7	0.86/0.81	61
		R: CTCCTGACCAAGGCAATATGT		175–207	6/7	0.75/0.93	
MSM1229	BV678272	F: ACCTGGGTGAGTCAACTTTAG	$(GT)_6(AT)_{11}$	122–140	15/8	**0.46/0.86**	63
		R: AAAGTTCCCACAGCTACTCAT		120	6/1	—	
MSM1230	BV678273	F: CACCAGACTCCCTTTTAATCACAT	$(GT)_{28}$	108–170	15/12	0.71/0.83	55
		R: TCATGGAGAATTTTGTTGTCAACT		154–156	6/2	0/0.48	
MSM1239	BV678278	F: GTTGCCATTGTCACGCCAGTA	$(CA)_{28}$	224–250	15/8	**0.47/0.81**	61
		R: TTTCTTCACGCCCGCTGATTA		240–246	6/4	0.17/0.74	
MSM1243	BV678663	F: GTTGCTGCTTTAGGTTGGACA	$(CA)_{18}$	222–244	15/6	0.79/0.81	61
		R: TTGTGTGAGCAATTAGAGCGA		224–230	6/2	0.67/0.48	
MSM1246	BV678290	F: CGAGAGCTGATTATGTGTGGTCAT	$(CA)_{30}$	214–238	15/8	**0.33/0.83**	61
		R: CATTAGCAGCAGGACCTGATGTAA		181–189	6/4	0.67/0.71	

repeats were screened. One library (SB-PE1) was 25% enriched and two (SB-PE2 and SB-PE7) were > 60% enriched.

Sequencing of clones was carried out using an ABI PRISM 3700 DNA Analyser (Applied Biosystems), and sequence analysis was performed with Vector NTI Suite 7.0 (Invitrogen). Sequences were aligned and primers were designed for unique, high-quality sequences with OLIGO version 6.0 software (Molecular Biology Insights).

Polymerase chain reaction (PCR) amplification of microsatellite loci was carried out in 10 μL reactions containing 1.0 μL DNA (~10 ng/μL), 2 mM $MgCl_2$, 48.2 μM of each dNTP (Promega), 1 μL 10× buffer (QIAGEN), 0.48 μM forward primer (Integrated DNA Technologies), 0.50 μM reverse primer with 5′ fluorescent label (Applied Biosystems) and 0.48 U HotStar *Taq* DNA polymerase (QIAGEN). Thermal cycling parameters consisted of 95 °C for 15 min, 35 cycles each at 94 °C for 30 s, annealing temperature for 30 s and 72 °C for 30 s, followed by 1 cycle of final elongation at 72 °C for 10 min. Amplification was performed either in multiplexed sets of two to four markers or singly. Amplified products were run on an ABI PRISM 3700 DNA analyser with GENESCAN 500 LIZ size standard, and alleles were identified using GENEMAPPER software version 3.0 (Applied Biosystems).

A total of 138 primer pairs from the SB-PE2 and SB-PE7 libraries were evaluated for polymorphism using a geographically diverse screening panel of DNA sampled from wild and captive-bred striped bass ($n = 15$) and white bass ($n = 6$). Two hybrids were included for evaluation of amplification. Fifteen additional markers from the SB-PE1 library were screened before the full panel was available. In all, 153 microsatellite loci were evaluated (GenBank Accession nos BV678169–BV678309; BV678652–BV678663).

Only four markers failed to reliably amplify in either species (GenBank Accession nos BV678253, –83, –89, and –97). Detailed information for 149 amplifying markers can be found in the primer database (http://tomato.bio.trinity.edu/home.html). For striped bass, 147 markers amplified successfully with a range of one to 17 alleles per locus and an average of 4.7 alleles per locus. In white bass, 133 markers amplified successfully with one to 10 alleles per locus (average 2.2 alleles per locus). Seventy-one markers were polymorphic in both species; 50 markers had ≥ 6 alleles in striped bass and 38 had ≥ 3 alleles in white bass. Of the 122 markers amplifying in both parent species and the hybrid (two alleles detected, one attributable to each parent species), 45 produced non-overlapping, species-specific allele size ranges (≥ 10 bp difference between parental species) and may be useful for detecting introgression.

Observed and expected heterozygosities, Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) were assessed using GENEPOP (http://wbiomed.curtin.edu.au/genepop/). Significance was evaluated after correction for multiple tests (Rice 1989). Overall average heterozygosity was 0.56 for striped bass and 0.45 for white bass. Significant deviations from HWE were observed for seven loci in striped bass. These deviations may indicate the presence of null alleles or result from pooling of samples from various geographical locations by species for analysis. No significant LD was detected. Data for a subset of the markers are presented in Table 1. These 29 markers amplified in both parents and in the hybrid and had expected heterozygosities ≥ 0.75 in striped bass; as such, these markers should prove especially useful for genetic evaluations in both wild and captive populations.

Contribution of numerous new microsatellite markers for *Morone* species provides necessary molecular tools for detailed genetic analysis of stock structure in wild populations and for selective breeding and linkage mapping in aquaculture.

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